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Effect of intermittent irradiation and fluence-response of 222 nm ultraviolet light on SARS-CoV-2 contamination

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ABSTRACT

Background: The effectiveness of 222 nm ultraviolet (UV) C light for disinfecting surfaces contaminated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been reported. The aim of this study was to evaluate the effect of the intermittent irradiation of 222 nm UVC on SARS-CoV-2 and the fluence-dependent effect of 222 nm UVC irradiation on SARS-CoV-2 inactivation.

Methods: We experimented with 5 min continuous and intermittent irradiation for 0.1, 0.05, 0.013, and 0.003 mW/cm^2 of 222 nm UVC to evaluate the differences in the effect of the continuous and intermittent irradiation of 222 nm UVC on SARS-CoV-2 inactivation. For intermittent irradiation, we followed the on-off irradiation cycles with every 10-s irradiation followed by a 380-s interval. Thereafter, we evaluated the effects of 0.1, 0.013, and 0.003 mW/cm^2 222 nm UVC irradiation on SARS-CoV-2 contamination at UV fluences of 1, 2, and 3 mJ/cm^2 at each irradiance.

Results: At each irradiance, no significant difference was observed in the log reduction of SARS-CoV-2 between continuous and intermittent irradiation. At each UV fluence, no significant difference was observed in the log reduction of SARS-CoV-2 among the three different irradiance levels.

Conclusion: There was no significant difference between continuous and intermittent irradiation with 222 nm UVC with regards to SARS-CoV-2 inactivation. Moreover, 222 nm UVC inactivates SARS-CoV-2 in a fluence-dependent manner. The efficacy of 222-nm UVC irradiation in reducing the contamination of SARS-CoV-2 needs to be further evaluated in a real-world setting.

1. Introduction

Coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is currently a global health issue. Studies show that SARS-CoV-2 remains active on plastic and steel surfaces for up to three days [1,2]. Furthermore, surfaces in hospitals that are treating COVID-19 patients were found to be contaminated by SARS-CoV-2 [3], thus suggesting the possibility of indirect transmission via surfaces. However, recent reports have shown the effectiveness of ultraviolet light (UV) irradiation for inactivating SARS-CoV-2 [4–6]. The effectiveness of 222 nm UVC light for disinfecting surfaces contaminated with SARS-CoV-2 has been reported [7]. In this previous study, the 222 nm UVC-emitting device, Care222™(Ushio Inc., Tokyo, Japan; Dimensions: 205 mm x 150 mm x 50 mm) was used and the effect of 0.1 mW/cm^2 222 nm UVC with multiple irradiation times on surfaces contaminated with SARS-CoV-2 was investigated [7]. When Care222™ was installed on the ceiling or wall, the UV irradiance of distant areas such as desks and floors was lower than that of a previous study setting [7]. Additionally, for use in an occupied space, Care222™ is used with the on–off intermittent irradiation mode with low UV irradiance or with a motion sensor mode to irradiate 222 nm UVC only when there are no individuals in the room. However, there are no published data on the required fluence and duration of low irradiance 222 nm UVC radiation for SARS-CoV-2

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inactivation. Furthermore, there are no data on the difference between the continuous and intermittent irradiation of 222 nm UVC on SARS-CoV-2 inactivation. The current study evaluated the effectiveness of 222 nm UVC intermittent irradiation (on-off irradiation cycles: 10 s irradiation followed by a 380 s interval) and fluence-dependence of 222 nm UVC irradiation for SARS-CoV-2 inactivation.

2. Materials and methods

2.1. Cells, virus, and $TCID_{50}$

SARS-CoV-2/JP/Hiroshima-46059T/2020 was used as the test virus. The cells used in this study and the experimental conditions, including the preparation of SARS-CoV-2-contaminated plates and the harvesting of the virus from plates, were the same as those described in a previous report [7]. The virus titer was determined using the standard 50 % tissue culture infectious dose ($TCID_{50}$) method and was expressed as $TCID_{50}$/mL [7]. Log_{10} $TCID_{50}$/mL reductions were calculated by comparing the log_{10} $TCID_{50}$/mL values recovered from plates after 222 nm UVC irradiation with those from control (non-irradiated) plates.

2.2. Irradiation condition with 222 nm UVC

The Care222™ (Ushio Inc., Tokyo, Japan) UVC-emitting device used in this study was a 222 nm Kr–Cl excimer lamp module. The lamp contains an optical filter that restricted spectra-emitting light ranging between 200 and 230 nm, of which the maximum output wavelength was 222 nm [7,8]. To evaluate the differences in the effect of the continuous and intermittent irradiation of 222 nm UVC on SARS-CoV-2 inactivation, we experimented with 5 min of continuous and intermittent irradiation for 0.1, 0.05, 0.013 and 0.003 mW/cm^2 of 222 nm UVC. The Care222™ was placed 24 cm above the surface of the plates, and the radiation irradiance at the surface of the plates was 0.1 mW/cm^2, as measured with an S-172/UIT250 UV meter (Ushio Inc., Tokyo, Japan). The 0.05, 0.013, and 0.003 mW/cm^2 of 222 nm UVC were almost the same irradiance as the 222 nm UVC irradiance at 50, 100, and 200 cm from Care222™, respectively. To conduct these experiments in a biosafety cabinet, the irradiation window was covered with a Teflon-based cover to reduce irradiance. The distance was adjusted to 0.05, 0.013, and 0.003 mW/cm as measured with an S-172/UIT250 UV meter. A spectroradiometer USR-45D (Ushio Inc., Tokyo, Japan) also confirmed that the Teflon-based cover did not affect the spectral distribution of ultraviolet light irradiated by Care222™. For 5 min of intermittent irradiation, the composition of the on–off irradiation cycles (10 s irradiation followed by a 380 s interval) was chosen on the basis of preliminary experiments. In the preliminary experiments, we tested 10, 30, and 60 s for on-time and their inactivating effect on SARS-CoV-2 was almost the same. Therefore, 10 s was chosen as on-time to evaluate the inactivating effect of intermittent irradiation with a greater number of on-off cycles. Since the off-time does not affect inactivation, 380 s was chosen as off-time for the convenience of the experiment. A programmable logic controller was built to automatically supply the on-off power into Care222™.

To evaluate the fluence–response of SARS-CoV-2 to 222 nm UVC irradiation, we evaluated the effects of 0.1, 0.013, and 0.003 mW/cm^2 222 nm UVC irradiation on SARS-CoV-2 contamination according to the time described in Table 1: UV fluences of 1, 2, and 3 mJ/cm^2 at each irradiance. For each experiment, the control plate was stored at room temperature until the end of UV exposure for treatment plates. All experiments were performed under visible light. All experimental results were reported as mean values across three replicates.

Table 1
Irradiation (treatment) time for evaluating the irradiance dependency of 222 nm UVC irradiation for inactivating SARS-CoV-2.

Irradiance (mW/cm^2)	Fluence (mJ/cm^2)		
	1	2	3
0.1	10 s	20 s	30 s
0.013	77 s	154 s	231 s
0.003	334 s	667 s	1000 s

2.3. Statistical analysis

Statistical analyses were performed using JMP 14.0 (SAS Institute Inc., Cary, NC, USA) to evaluate the differences in infectious viral titers. P-values were computed using a two-sided independent-samples *t*-test. $P < 0.05$ was considered statistically significant.

3. Results

Fig. 1 and Table 2 shows a comparison of the $TCID_{50}$ assay results of the continuous and intermittent irradiation of 222 nm UV light on SARS-CoV-2 for 5 min. At each irradiance, no significant difference in the log reduction of SARS-CoV-2 was observed. Fig. 2 and Table 3A shows the results of the $TCID_{50}$ assay for evaluating the effect of 222 nm UVC irradiation on SARS-CoV-2 for irradiance values of 1, 2, and 3 mJ/cm^2 at different intensities. At each UV fluence, there was no significant difference in the log reduction of SARS-CoV-2 among the three different irradiance levels (0.1 vs. 0.013, 0.1 vs. 0.003, and 0.013 vs. 0.003 mW/cm^2) (Table 3B).

4. Discussion

This study was not a noninferiority trial or equivalence trial, and the sample size was small. However, the error was small, and there was no significant difference in the confidence interval of data among each experiment (Tables 2, 3A, and 3B). Therefore, we concluded that there was no significant difference in the inactivating effect on SARS-CoV-2 between the continuous and intermittent irradiation of 222 nm UVC when the total irradiation time was the same. In addition, we concluded that at each UV fluence, there was no significant difference in the log reduction of SARS-CoV-2 among the three different irradiance levels (0.1 vs. 0.013, 0.1 vs. 0.003, and 0.013 vs. 0.003 mW/cm^2). This result suggested that the intermittent irradiation of 222 nm UVC has an additive effect on inactivating SARS-CoV-2 and is fluence-dependent. Presumably, if the on-time is reduced continuously, for the same off time, the inactivating effect of 222 nm UVC on SARS-CoV-2 will be reduced.

A variety of organisms possess molecular mechanisms to compensate for the UV-induced DNA damages. Photoreactivation is one of most widely studied repair mechanisms which uses an enzyme called photolyase and light energy [9]. Photoreactivation causes problems for large-scale UVC inactivation of microorganisms when the treated object such as wastewater or drinking water are exposed to sunlight. However, this result may indicate the absence of SARS-CoV-2 photoreactivation in this experimental environment. Previous studies have demonstrated the absence of photoreactivation in most viruses owing to the lack of biological processes such as enzymes and cellular functions that orchestrate photoreactivation [10]. However, a recent report showed that a few viruses such as T1 and PRD1 might undergo photoreactivation via the host bacteria. By contrast, no photoreactivation was observed in MS2 even with hosts [11]. Moreover, we showed that 222 nm UVC inactivates SARS-CoV-2 in a fluence-dependent manner and not in an irradiance-dependent manner, which is consistent with a previous report on other viruses [12]. This result suggested that SARS-CoV-2 can be inactivated by long term irradiation with a low irradiance of 222 nm UVC at a location away from Care222™, such as a desk or floor. In occupied space, 222 nm UVC is irradiated by Care222™ with the on-off intermittent irradiation mode or a motion sensor within the current exposure limits recommended by the American Conference of Governmental Industrial Hygienists for not posing a health risk to humans. The

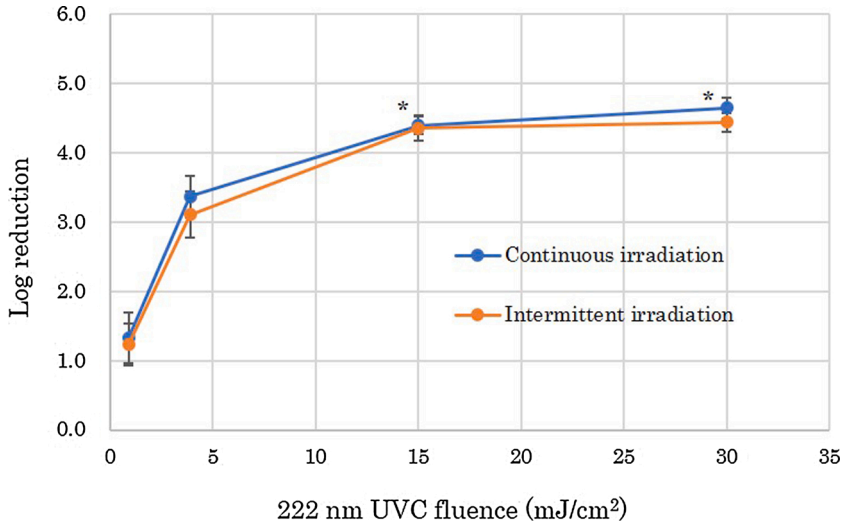

Fig. 1. Comparison of the continuous and intermittent irradiation of 222 nm UVC light on SARS-CoV-2 for 5 min. * The titers of SARS-CoV-2 in the treatment plates were undetectable based on the $TCID_{50}$ assay.

Table 2
The detailed comparison of the continuous and intermittent irradiation of 222 nm UVC light on SARS-CoV-2 for 5 min.

		Log reduction					
		Continuous irradiation		Intermittent irradiation			
Irradiance (mW/cm^2)	Fluence (mJ/cm^2)	Mean (SD)	95 % CI	Mean (SD)	95 % CI	Mean difference (95 % CI)	P-value
0.1	30	>4.65 (0.36) *	N/A	>4.40 (0.30) *	N/A	N/A	N/A
0.05	15	>4.40 (0.30) *	N/A	>4.36 (0.33) *	N/A	N/A	N/A
0.013	3.9	3.37 (0.13)	3.11–3.63	3.11 (0.19)	2.84–3.37	0.26 (-0.62–0.11)	0.119
0.003	0.9	1.33 (0.15)	1.09–1.57	1.23 (0.14)	0.99–1.46	0.10 (-0.43–0.23)	0.441

SD, standard deviation; CI, confidence interval; N/A, not available.
* The titers of SARS-CoV-2 in the treatment plates were undetectable on the basis of the $TCID_{50}$ assay.

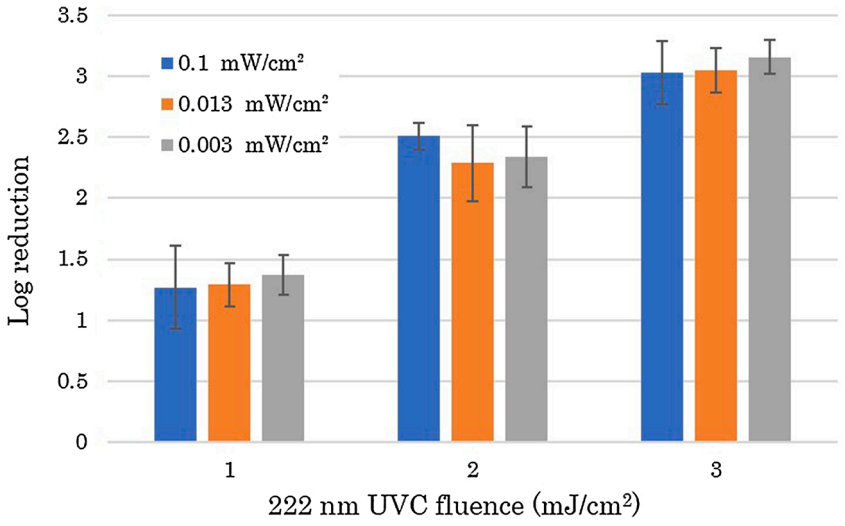

Fig. 2. $TCID_{50}$ assay for evaluating the effect of 222 nm UVC irradiation on SARS-CoV-2 for 1, 2, and 3 mJ/cm^2 at different irradiances.

results of this study also suggest that intermittent irradiation with a low irradiance 222 nm UVC and continuous irradiation with a high-irradiance 222 nm UV can inactivate SARS-CoV-2 when the total UV fluence is the same.

In conclusion, we demonstrated that there was no significant difference in the inactivation effect of continuous and intermittent irradiation of 222 nm UVC on SARS-CoV-2. Moreover, 222 nm UVC inactivates SARS-CoV-2 in a fluence-dependent manner. The efficacy of 222-nm UVC irradiation in reducing the contamination of SARS-CoV-2 needs to be further evaluated in a real-world setting.

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Table 3A
The detailed data of $TCID_{50}$ assay for evaluating the effect of 222 nm UVC irradiation on SARS-CoV-2 for 1, 2, and 3 mJ/cm^2 at different irradiances.

Irradiance (mW/cm^2)	Fluence (mJ/cm^2)					
	1		2		3	
	Mean (SD)	95 % CI	Mean (SD)	95 % CI	Mean (SD)	95 % CI
0.1	1.27 (0.34)	0.43–2.12	2.51 (0.11)	2.22–2.80	3.03 (0.26)	2.40–3.67
0.013	1.29 (0.18)	0.82–1.76	2.29 (0.31)	1.51–3.08	3.05 (0.18)	2.60–3.50
0.003	1.37 (0.16)	0.98–1.77	2.34 (0.25)	1.71–2.97	3.16 (0.14)	2.81–3.52

SD, standard deviation; CI, confidence interval.

Table 3B
Results of the statistical analysis of the three different irradiance levels of each UVC fluence.

	Fluence (mJ/cm^2)					
	1		2		3	
	Mean difference (95 % CI)	P-value	Mean difference (95 % CI)	P-value	Mean difference (95 % CI)	P-value
0.1 vs. 0.013	0.017 (-0.61–0.64)	0.94	0.19 (-0.75–0.32)	0.33	0.18 (-0.48–0.51)	0.93
0.1 vs. 0.003	0.22 (-0.50–0.70)	0.67	0.16 (-0.61–0.27)	0.36	0.17 (-0.34–0.61)	0.48
0.013 vs. 0.003	0.14 (-0.31–0.48)	0.59	0.05 (-0.60–0.70)	0.84	0.12 (-0.25–0.49)	0.43

% CI, 95 % confidence interval.

Declarations of Competing Interest

None.

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